

CLEAN COPY OF SUBSTITUTE PAGES 11-13 -- 09/786,926

GT-3') (SEQ ID NO:5) , R3 (5'-CG-GGA-TCC-GAA-RGY-RTA-SAD-SAD-RGG-RTT-3') (SEQ ID NO:6) . Cycle: 94°C, 60 sec.; 48-63°C, 30 sec.; 72°C, 90 sec.; 35 cycles. For the amplification of the 3' and 5' ends of the human edg6 cDNA, a RACE-PCR was carried out with the following primers: 5'-hGSPRT (5'-TTG-GAG-CCA-AAG-ACG-TCG-GCC-3') (SEQ ID NO:7) , 5'-HGSP1 (5'-AGG-CAG-AAG-AGG-ATG-TAG-CGC-3') (SEQ ID NO:8) , 5'-HGSP2 (5'-GCG-CTC-CCC-TGC-AGT-GAA-GAG-3') (SEQ ID NO:9) , 3'-hGSP1 (5'-AGT-GAC-CTG-CTC-ACG-GGC-GCG-3') (SEQ ID NO:10) , 3'-hGSP2 (5'-CTCTTCACTGCAGGGGAG-CGC-3') (SEQ ID NO:11) . The reactions were carried out according to the protocol of M.A. Frohman (Frohman, 1995). The amplification of the 5' end of the murine edg6 cDNA was likewise done with the help of the RACE-PCR with the following primers: 5'-mGSPRT (5'-CTC-ACC-TCG-TCT-GGG-AGG-GCC-TGC-3') (SEQ ID NO:12) , 5'-mGSP1 (5'-TGG-GCA-ACT-GGC-TGG-TCC-AAG-CTC-3') (SEQ ID NO:13) , 5'-mGSP2 (5'-GCC-TCG-GGC-CCA-GAT-CCT-CCA-GGG-GTG-CTG-CGG-ACG-CTG-GAA-ATG-CTG-G-3') (SEQ ID NO:14). Before, as described above, a reverse transcription was done with 10 µg of overall RNA of the murine cell line 18. The 5'-mGSP2 primer contains a part of the myc-epitope sequence for further experiments. The primers were selected on the basis of the murine EST sequence of the cDNA clone val6c04.r1 (Gene Bank entry no. AA254425), which has a high homology with the 3' end of the coding human edg6 cDNA. The reactions were also carried out according to the protocol of M.A. Frohman (Frohman, 1995) with an additional cleaning step by means of "MicroSpin S-400 HR" columns of the firm of Pharmacia Biotech on the basis of the supplied protocol following the 5' polyadenylation reaction. Further, 10 µl of undiluted presentation DNA were used for both amplifications of the RACE-PCR. The murine edg6 cDNA fragment, which was used in the Northern blot as a radioactively marked sample, was amplified as described above through the reverse transcriptase polymerase chain reaction from an overall RNA preparation of the murine cell line 18 with 25 pmol each of the 3' primer (5'-CCA-CGT-CCT-CCT-GCC-CGC-CGC-3') (SEQ ID NO:15) and 25 pmol of the 5'-mGSP2 primer (see above). Cycle: 94°C, 60 sec.; 50°C, 60 sec.; 72°C,

CLEAN COPY OF SUBSTITUTE PAGES 11-13 -- 09/786,926

90 sec.; 35 cycles. The amplification of the genomic 3' sequence of the human *edg6* was done by means of PCR from 400 ng HEp2 genomic DNA with 25 pmol of the 3'-hGSP2 primer (see above) and 25 pmol of the CA primer (5'-CCA-CTT-CCC-GCA-ACG-CCC-AGA-3') (SEQ ID NO:16). Cycle: initial denaturing, 95°C, 5 min.; 95°C, 30 sec.; 60°C, 30 sec.; 72°C, 90 sec.; 30 cycles.

Cloning and sequencing

The cDNA fragments of the PCR reactions with the degenerated primers were cloned into the pZerO-2 vector of the firm of Invitrogen to Bam HI Verdau. The human *edg6* RACE-PCR products were cloned into the same vector to HIND III/Pst I Verdau. They were ligated to a full-length clone at the Pst I interface. The murine *edg6* 5'-RACE-PCR product was cloned into the pZerO-2 vector to HIND III/Eco RV restriction. For this, the RACE-PCR product was HIND III-digested after a T4-polymerase reaction. The human cDNA fragment for the radioactive marking was isolated to Pst I/Aat II-restriction of the full-length clone (bp 438-842). The amplified murine cDNA fragment (bp 328-637) was cloned into the Apa I cut pZerO-2 vector. This fragment was used as a sensor in Northern blot after radioactive marking. All the fragments were sequenced with the "Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP" of the firm of Amersham International and analysed with the help of the Li-Cor sequencer of the firm of MWG Biotech according to the supplied protocols.

Construction, expression and FACS analysis of the myc-epitope marked human EDG6 receptor

The construction of the C-terminal myc-epitope marked human EDG6 receptor and its expression in HEK293 cells and its analysis by means of throughflow cytometry was done as described (Emrich et al., 1993).

CLEAN COPY OF SUBSTITUTE PAGES 11-13 -- 09/786,926

Computer analyses

Sequence comparisons, database research and statistical calculations were done with the help of HUSAR package V4.0 at the German Cancer Research Centre in Heidelberg and with the ClustalX V1.62b PC programme.